Research report

Corticospinal tract transection reduces H-reflex circadian rhythm in rats

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Abstract

In freely moving rats and monkeys, H-reflex amplitude displays a marked circadian variation without change in background motoneuron tone. In rats, the H-reflex is largest around noon and smallest around midnight. The present study evaluated in rats the effects on this rhythm of calibrated contusions of mid-thoracic spinal cord and mid-thoracic transection of specific spinal cord pathways. In 33 control rats, rhythm amplitude averaged $29.0(\pm 2.6 \text{ S.E.})\%$ of H-reflex amplitude. Contusion injuries at T8–9 that destroyed 53–88% of the white matter significantly reduced the rhythm to $18.9(\pm 2.4)\%$ of H-reflex amplitude. Transection of the ipsilateral lateral column, which contains the rubrospinal, vestibulospinal, and reticulospinal tracts, or bilateral transection of the dorsal column ascending tracts did not affect rhythm amplitude or phase. In contrast, bilateral transection of the main corticospinal tract significantly reduced the rhythm to $14.7(\pm 6.6)\%$. These results indicate that the H-reflex circadian rhythm depends in part on descending influence from the brain and that this influence is conveyed by the main corticospinal tract. © 2002 Elsevier Science B.V. All rights reserved.

Theme: Motor systems and sensorimotor integration

Topic: Reflex function

Keywords: Circadian rhythm; Diurnal rhythm; H-reflex; Spinal cord injury; Corticospinal tract; Rat

1. Introduction

The spinal stretch reflex (SSR) is the simplest behavior of the vertebrate CNS. It is produced primarily by the wholly spinal and largely monosynaptic pathway consisting of the Ia afferent, the α-motoneuron, and the synapse between them [5,36]. Studies in monkeys and rats have revealed a prominent circadian rhythm in the SSR and in its electrical analog, the H-reflex [11,13,23,60,61]. This rhythm is evident even when background motoneuron tone (measured as electromyographic (EMG) activity) does not change. In monkeys, the reflex is largest around midnight and smallest around noon, while in rats, which are nocturnal, it is largest around noon and smallest around midnight. Thus, in both species the reflex is largest during the part of the day when the animal is usually less active and smallest during the part when it is usually most active.

Circadian rhythms are apparent in many aspects of mammalian CNS function and their neuronal mechanisms are under investigation [1,6,32,37–39,49,64]. The prominent rhythm evident in the SSR and H-reflex is of interest first because it indicates that the CNS activity underlying motor performance varies with time of day, and second because the simplicity and accessibility of the spinal reflex pathway may provide an excellent opportunity to locate and define its underlying mechanisms.

Spinal cord reflex pathways normally operate under the control of descending activity from the brain. These descending pathways exert short-term task-dependent control and also exert long-term control that shapes spinal cord function during early development and during skill acquisition later in life and contributes to the reflex abnormalities associated with spinal cord injury and other disorders (Ref. [2,4,7,21,22,24–28,35,42,50–52,54,58,63]; see Ref. [62] for review). Furthermore, spinal cord...
injury in humans disturbs the circadian rhythm normally seen in blood pressure [8,40,41]. Thus, it is possible that descending pathways contribute to the circadian rhythms in the SSR and H-reflex. On the other hand, sensorimotor cortex evoked potentials that accompany these spinal reflexes display a circadian rhythm that is not in phase with the rhythm seen in the reflexes [23]. This suggests that the spinal and supraspinal rhythms may have independent mechanisms.

The present study used data gathered in studies of long-term descending control of the rat soleus H-reflex [10,17,18] to explore the role of descending control in the H-reflex circadian rhythm. It asked two questions. First, does interaction between the brain and spinal cord have a role in this rhythm? This was addressed by measuring the effects on the rhythm of contusions of mid-thoracic spinal cord. Second, if an interaction does occur, which spinal cord pathways contribute to it? This was addressed by measuring the effects on the rhythm of mid-thoracic transection of specific spinal cord pathways. The results indicate that descending influence from supraspinal structures contributes to circadian variation in the rat H-reflex. They also indicate that the main corticospinal tract conveys this influence.

2. Materials and methods

Animal preparation and data collection methods have been described in detail elsewhere [10–13,17,18]. They are summarized here. All procedures satisfied the ‘Guide for the Care and Use of Laboratory Animals’ of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, DC, 1996) and had been reviewed and approved by the Institutional Animal Care and Use Committees of the Wadsworth Center and the Ohio State University.

2.1. Measurement of H-reflex, M response, and background EMG

Forty-four female Sprague–Dawley rats weighing 200–330 g were implanted under general anesthesia (intraperitoneal ketamine HCl (80 mg/kg) and xylazine (10 mg/kg)) with chronic stimulating and recording electrodes in the right leg. To elicit the H-reflex, a silicone rubber nerve cuff containing a pair of stainless steel multi-stranded fine-wire electrodes was placed on the posterior tibial nerve just above the triceps surae branches. To record soleus EMG activity, a pair of fine-wire electrodes with the final 0.5 cm bare were placed in the right soleus muscle. The Teflon-coated wires from the nerve cuff and the muscle traveled subcutaneously to a connector plug mounted on the skull with stainless steel screws and dental cement. Seven to 10 days after implantation surgery, each rat was tested with nerve-cuff stimulation to ensure that an H-reflex was present at M response (i.e. direct muscle response) threshold.

Data collection began at least 10 days after electrode implantation. Throughout data collection, the animal was housed singly in a standard rat cage with a 40-cm flexible cable attached to the skull plug. The cable, which allowed the animal to move freely about the cage, carried the wires from the electrodes to an electronic swivel above the cage, from whence they passed to an EMG amplifier and a nerve-cuff stimulation unit. All animals had free access to food and water. Animal well-being was carefully checked several times each day, and body weight was measured weekly. Laboratory lights, controlled by a timer, were bright during the day (06:00–21:00 h) and dim during the night (21:00–06:00 h). Room temperature was kept constant (20 °C). Animals remained healthy and active throughout data collection.

Soleus EMG was monitored continuously by computer. If the absolute value of background (i.e. ongoing) EMG (i.e. equivalent to the full-wave rectified value) remained within a defined range for a randomly varying 2.3–2.7 s period, a stimulus pulse (typically 0.5 ms in duration) just above M response threshold was delivered by the nerve cuff. Pulse amplitude was automatically adjusted after each trial so as to maintain constant M response amplitude throughout data collection. The computer digitized and stored soleus EMG absolute value for 50 ms following the stimulus. It also digitized and calculated the amplitude of soleus background EMG (i.e. average absolute value of EMG for the 50 ms immediately prior to stimulation) and provided data summaries for every 3-h and every 24-h period. Each summary included the number of trials, average amplitude of soleus background EMG, average course of EMG amplitude for the 50-ms post-stimulus period, average amplitude in the M-response interval (typically 1.5–4.0 ms after stimulation) and average amplitude in the H-reflex interval (typically 5.5–10.0 ms after stimulation). Because the goal was to measure the H-reflex throughout the day without change in background EMG or M response amplitude, the data from those few 3-h periods in which background EMG was not within 10% of its daily average and/or M response amplitude was not within 25% of its daily average were excluded from analysis [11]. In the course of its daily activity, the animal normally satisfied the background EMG requirement, and therefore received nerve cuff stimulation, 2500–9300 times per day. H-reflex amplitude was measured as average absolute EMG amplitude in the H-reflex interval minus average background EMG amplitude, and M response amplitude was measured as average absolute EMG amplitude in the M response interval minus average background EMG amplitude.

2.2. Spinal cord contusions

Twenty-three rats received a contusion injury to the thoracic spinal cord (SCC rats) at the Ohio State Universi-
ty as previously described [17,18,55,56]. Briefly, each rat was anesthetized with an intraperitoneal injection of ketamine HCl (80 mg/kg) and xylazine (10 mg/kg), a partial dorsal laminectomy was carried out at the T8–T9 vertebral junction, and over a 23-ms period a piston depressed the dura over the dorsal surface of the spinal cord in the midline either 0.7 mm (mild contusion, 15 rats) or 0.9 mm (medium contusion, eight rats). Previous studies have shown that the severity of injury is correlated to displacement model in this model. The wound site was closed, and postoperative care was administered as previously described [17,18]. At least 10 days after injury, each rat was implanted with recording and stimulating electrodes at the Wadsworth Center as described above. Beginning at least 27 days after injury, data were collected from each rat for 10–32 days.

2.3. Spinal cord pathway transections

In rats, the dorsal column contains the main corticospinal tract (CST) and the dorsal ascending tract (DA) conveying input from proprioceptors and skin receptors [19,20,33,34,44,45,53,57]. The lateral column (LC) contains the rubrospinal, vestibulospinal, and reticulospinal tracts and a variety of ascending tracts [31,33,34,57,65,66].

For each rat scheduled to receive a pathway transection, data were collected for 10–22 days to determine the animal’s initial (i.e. pre-transsection) H-reflex circadian rhythm. Then, the rat was subjected under anesthesia to a bilateral CST transection (seven CST rats), a bilateral DA transection (six DA rats), or a right LC transection (eight LC rats) at T8–9 by electrocautery. (As noted above, the right soleus H-reflex was studied in all rats.) Transection procedures and postoperative care were as previously described [10,15,16]. Data collection continued for at least 20 days after the transection.

2.4. Animal perfusion and histology

At the end of study, which for most rats was after subsequent exposure to the H-reflex operant conditioning protocol [15–18], each rat was given an overdose of sodium pentobarbital (i.p.) and perfused through the heart. The spinal cord was removed and blocks encompassing the lesion from the contusion injury or the transection were embedded in paraffin. Transverse 10- to 20-μm-thick serial sections were cut from the paraffin-embedded blocks and stained with Luxol fast blue (for myelinated fibers) and 0.1% cresyl violet (for Nissl substance). Sections encompassing the lesion were assessed as previously described to determine the location and size of the spinal cord injury or the transection [10,17,18]. For rats with spinal cord contusions, the area of remaining white matter was calculated as percent of the white matter area of a reference section from thoracic spinal cord 3.5–4.0 mm rostral to the injury epicenter. (In agreement with Olby and Blakemore [43], white matter area at a level rostral to the rostral limit of the lesion was found to be comparable to that of normal rats.) For CST and DA rats, the area of CST or DA remaining was measured as percent of the area of that structure 2–5 mm rostral to the rostral limit of the lesion. For LC rats, the area of right LC remaining was measured as percent of the left LC. Multiple examples of contusion injuries and pathway transections have been published previously (Fig. 1 in Refs. [15–17], Fig. 2 in Ref. [10], and Fig. 4 in Ref. [18]).

2.5. Data analysis

For each 3-h summary period, H-reflex amplitude, M response amplitude, and background EMG were calculated as percent of the day’s average value for each rat. Number of trials was calculated as percent of the day’s total. For SCC rats, results were averaged for the last 10 days of data collection for this study (which ended 37–270 days after injury). For CST, DA, and LC rats, average values were determined for the last 10 days prior to transection and for days 11–20 after transection. The amplitude of the circadian variation in the H-reflex was calculated as the average H-reflex amplitude for the two 3-h periods with the highest H-reflex amplitudes minus average H-reflex amplitude for the two 3-h periods with the lowest H-reflex amplitudes and expressed in percent of average H-reflex amplitude for the day. The periods with the two highest amplitudes were always adjacent (usually 10:00–13:00 h and 13:00–16:00 h), and so were the two periods with the two lowest amplitudes (usually 22:00–01:00 and 01:00–04:00 h).

To determine the effect of spinal cord contusions on the circadian rhythm, the rhythm amplitudes for the SCC rats were compared by t-test to those of 33 control rats [10–13,15,16]. To determine the effect of CST, DA, or LC transection, post-transection and pre-transection values from the individual rats were compared by paired t-test.

3. Results

As previously described [10,15–18], spinal cord contusion or pathway transection produced a transient hindlimb paralysis (both hindlimbs for SCC, CST, and DA rats and right only for LC rats) that abated within 5 days for SCC rats and within 3 days for CST, DA, and LC rats. For all rats, quadrupedal locomotion returned within 3 weeks. Bladder function, absent immediately after injury, returned over 0–7 days. After the immediate post-traumatic period, all rats remained healthy and active until perfusion. Average weight increased from 294 (±41 S.D.) g at contusion or transection to 352 (±40 S.D.) g at perfusion. After perfusion, soleus muscle weights (measured as percent of body weight) were symmetrical and did not differ significantly from those of normal rats.

In SCC rats, the white matter remaining at the lesion epicenter averaged 28(±8 S.D.)%, and ranged from 12 to 47% of normal white matter area for mid-thoracic spinal
cord. The rats with mild (0.7-mm) contusion showed greater white matter remaining (31±11 S.D.%; range: 12–47) than rats with medium (0.9-mm) contusion (18±6 S.D.%; range: 12–29). The difference was statistically significant (P=0.007, t-test). The distribution of residual white matter with a normal Luxol fast blue appearance varied between SCC animals. All 23 SCC rats had some residual white matter in the lateral and ventral peripheral rim. Seven did not show any remaining dorsal column tissue. Fifteen appeared to have some preservation of DA, but not CST. Only one rat had apparently good preservation of DA and CST. However, due to the tissue shrinkage and distortion caused by the contusion and/or the tissue processing (see Fig. 1 [17] and Fig. 4 [18]), it was not possible to determine with confidence which tract or tracts were preserved.

In six of the seven CST rats, the CST was completely absent bilaterally. In the remaining rat, 54% of the right CST and 20% of the left CST remained. CST rats also sustained some loss of left LC and/or DA due to the contralateral approach of the cauteterizer [10]. They retained all of the right LC, 65±29 S.D.%; range 32–100%) of the left LC, 53±22% (range 18–78%) of the right DA, and 33±41% (range 0–98%) of the left DA. In the six DA rats, 9±12% (range 0–30%) of the right DA, 11±13% (range 0–32%) of the left DA, and all of the CST and LC remained. In the eight LC rats, 10±10% (range 0–28%) of the right LC, and all of the DA, CST, and left LC remained.

Fig. 1 summarizes the results for normal and SCC rats, and for CST, DA, and LC rats before and after transection. It shows H-reflex, M response, and background EMG amplitudes (in percent of day’s average) and number of trials (in percent of day’s total) for each 3-h period. The vertical axis scale is the same for H-reflex, M response, and background EMG amplitudes to facilitate their comparison. M response and background EMG amplitudes, controlled by the protocol (see Methods), changed little during the day. Trial number was greatest at night. Neither contusion nor CST, DA, or LC transection had any detectable effect on the slight circadian variations in M response and background EMG amplitudes, or on the

![Fig. 1. Average (±S.E.) values of H-reflex, M response, and background EMG (in percent of day’s average) and average (±S.E.) number of trials (in percent of day’s total) for each 3-h period for normal rats (dotted, left-most graphs) and SCC rats (solid, left-most graphs), and for CST, DA, and LC rats before (dotted) and after (solid) transection.](image-url)
Table 1: Average ± S.E. (%) H-reflex circadian rhythm amplitudes for normal rats (parentheses) and SCC rats, and for CST, DA, and LC rats before and after transection.

<table>
<thead>
<tr>
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<th>SCC Rats</th>
<th>CST Rats</th>
<th>DA Rats</th>
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<tr>
<td>Before</td>
<td>29.0±2.6</td>
<td>30.8±6.8</td>
<td>27.2±7.1</td>
<td>28.9±6.1</td>
</tr>
<tr>
<td>After</td>
<td>18.9±2.4*</td>
<td>14.7±6.6*</td>
<td>25.2±7.2</td>
<td>32.2±8.6</td>
</tr>
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An asterisk indicates a significant decrease from control (i.e. SCC rats, P = 0.005 by t-test) or from pre-transection values (i.e. CST rats, P = 0.02 by paired t-test). Data for normal rats are from 33 control rats from other studies [10–13,15,16].

Circadian variation in number of trials. H-reflex amplitude displayed a prominent circadian rhythm, averaging 29% in normal rats. SCC or CST transection reduced rhythm amplitude, while LC or DA transection did not do so. Table 1 summarizes the findings in regard to lesion effects on rhythm amplitude. Rhythm amplitude was markedly and significantly reduced in SCC rats and in CST rats. In SCC rats, rats with medium contusions showed a greater decrease in rhythm amplitude than those with mild contusions. The circadian rhythm amplitude averaged 12(±10 S.D.)% in rats with medium contusions, while it averaged 22(±11 S.D.)% in rats with mild contusions. The difference was statistically significant (P = 0.03, t-test). However, neither group showed a significant correlation between decrease in rhythm amplitude and amount of white matter lost (P > 0.5 for each). While rhythm amplitude was reduced in SCC and CST rats, rhythm phase was not affected. In contrast, DA or LC transection had no detectable effect on rhythm amplitude or phase. It should be noted that the rhythm amplitude of the one SCC rat that had good preservation of DA and CST (Fig. 1A in Ref. [17]) was 33.7%, very similar to the average for normal rats.

Fig. 2 illustrates the effect of CST transection with average post-stimulus EMG traces for the 3-h periods with the maximum and minimum H-reflex amplitudes from a CST rat for a day prior to transection and for the 20th day after transection. Background EMG and M response amplitudes are comparable in all four traces. Prior to transection, the H-reflex is much larger at 10:00–13:00 h than at 22:00–01:00 h. After transection, the H-reflexes for these two periods differ much less in amplitude.

4. Discussion

This study explored the effects of spinal cord contusions and specific spinal cord pathway transections on the H-reflex circadian rhythm of the rat soleus muscle. The H-reflex circadian rhythm was significantly decreased after spinal cord contusion or CST transection. Thoracic spinal cord contusions that destroyed an average of 73% of the white matter or destruction of the main corticospinal tract reduced the rhythm to about half of its normal amplitude, but did not affect its phase. In contrast, transection of the dorsal column ascending tract or transection of the ipsilateral lateral column did not affect rhythm amplitude or phase. The lateral column contains the rubrospinal, vestibulospinal, and reticulospinal tracts [31,33,34,57,65,66]. Because these tracts are almost completely ipsilateral at T8–9, it is unlikely that the contralateral LC accounted for preservation of the rhythm in LC rats. In CST rats, the adjacent DA and left LC pathways often sustained significant damage. However, it is unlikely that this collateral damage contributed significantly to the reduction in the rhythm in CST rats because CST, DA, and LC are all largely or totally ipsilateral in the thoracic spinal cord, because DA or LC transection alone had no discernible...
influence on the rhythm, and because the extent of collateral damage to left LC or right or left DA in CST rats did not correlate with the magnitude of rhythm reduction (P > 0.4 for each). Finally, collateral damage to adjacent areas of mid-thoracic gray matter would have been unlikely to affect the function of soleus motoneurons (which are located in the lumbar spinal cord) and their associated segmental pathways.

The decrease in H-reflex circadian rhythm in rats after spinal cord contusion or CST transection is not attributable to change in background muscle tone or strength of stimulation (reflected in M response amplitude). Background EMG and M response amplitude displayed only small circadian variations, and, most important, these variations were not affected by contusion or by CST, LC, or DA transection (P > 0.24 for each).

The circadian rhythms in spinal stretch reflex and H-reflex in monkeys [23,60,61] and rats [11] indicate that the neuronal activity underlying motor performance varies with time of day. The present study demonstrates that descending influence from the brain contributes to this rhythm, and that this influence is conveyed by the main CST. The H-reflex is produced primarily by the wholly spinal pathway consisting of the Ia afferent, the α-motoneuron, and the synapse between them [5,36]. Since the rat lumbar spinal cord contains no or very few direct CST-to-motoneuron connections [57], the CST influence that contributes to the rhythm probably acts through spinal interneurons that synapse on the motoneuron or provide presynaptic inhibition to the Ia synaptic connection [3,7,29].

As noted above, in monkeys the amplitude of the cortical somatosensory evoked potential evoked by the same stimulus that evokes the H-reflex also displays a circadian rhythm [23]. This rhythm is not in phase with the H-reflex rhythm. This suggests the presence of an additional or more complex central mechanism. At the same time, the present results suggest that the H-reflex circadian rhythm is not completely dependent on descending influence. Even in the five SCC rats in which only 12–15% of the white matter remained, rhythm amplitude was 16.1 (± 3.5 S.E.)%; and in the six CST rats in which the CST was completely destroyed, rhythm amplitude averaged 14.7 (± 7.8)%. The remaining rhythm may reflect a neuronal mechanism intrinsic to the spinal cord and/or a humoral mechanism [30,46–48]. On the other hand, ventral column (VC) descending pathways may also play a role in the remaining soleus H-reflex rhythm. The rat VC does contain important modulatory systems, and the minor CST as well [57].

Operant conditioning of the H-reflex in freely moving rats is a new model for studying long-term supraspinal control over spinal cord function (Refs. [15–18]; see Ref. [59] for review). Motivated by food reward, rats can gradually increase or decrease the soleus H-reflex [12,14]. This adaptive change occurs gradually over weeks. Animals can nearly double (up-conditioning mode) or almost halve (down-conditioning mode) H-reflex amplitude without change in background EMG or M response amplitude. Recent studies indicate that spinal cord contusions impair conditioning and that the degree of impairment correlates with the amount of white matter lost [17,18]. In the present study, rats with medium spinal cord contusions have greater white matter loss and greater decrease in H-reflex rhythm amplitude than those with mild spinal cord contusions. However, in neither SCC group was a significant relationship found between the decrease in H-reflex rhythm amplitudes and the amount of white matter lost. This absence of correlation is probably attributable to the considerable variation among normal rats in rhythm amplitude. It may also be attributable to the fact that the descending influence from the brain that contributes to the H-reflex rhythm is conveyed by the main CST. On average, the main CST comprises less than 3% of the total white matter of T8–9. In addition, it is located in a region of the spinal cord that is susceptible to wide variation in damage following mild contusions. Thus, the amount of white matter lost after contusion injury may not correlate closely with the amount of CST lost.

Pathway-specific transection studies indicate that operant conditioning of the H-reflex depends on the main CST and does not depend on the DA or the ipsilateral LC [9,15,16]. Thus, in addition to contributing to the circadian variation in the H-reflex, the CST is also essential for long-term adaptive changes in the H-reflex. On the other hand, the circadian rhythm and operantly conditioned change do not appear to interact with each other: in both rats and monkeys rhythm amplitude (as percent of average daily amplitude) and phase do not change when the H-reflex gradually increases in response to up conditioning or decreases in response to down conditioning, and neither rats nor monkeys alter daily performance schedules so as to use the rhythm to increase reward probability [14,60].

Intracellular studies of synaptic input to cat motoneurons indicate that temperature affects EPSP and IPSP amplitudes [46,47]. Amplitudes are higher at lower temperatures, and this appears to be attributable to an inverse relationship between temperature and motoneuron input resistance. Thus, circadian variation in body temperature might affect motoneuron responses in the rat. The requirement of the protocol that background EMG fall in a defined range prior to H-reflex elicitation would have prevented circadian change in motoneuron responsiveness from markedly affecting the tonic background EMG. However, such a circadian change could affect the motoneuron response to the Ia afferent stimulus that elicits the H-reflex, and thus might contribute to the circadian rhythm in the H-reflex.

In monkeys, the circadian rhythm in the biceps brachii SSR has a detectable functional effect: the elbow extension produced by a sudden perturbation is greater around noon when the SSR is smaller, and smaller around midnight.
when the SSR is larger [60]. This suggests that the circadian rhythm in the rat soleus H-reflex also has functional significance. Its existence indicates the CNS activity underlying a fixed motor performance differs with time of day. The present study provides new insight into the complex interactions between supraspinal and spinal cord structures that underlie motor control.

In summary, the present study implies that H-reflex circadian regulation is mediated by descending corticospinal tract function superimposed on an intrinsic spinal rhythm. These results provide a clear example of one mechanism whereby the brain contributes by a specific descending pathway to the modulation of segmental motor function over the course of a day.

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